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Genotypic Resistance Testing Creates New Treatment Challenges: Two Cases of Oxacillin-Susceptible Methicillin-Resistant *Staphylococcus aureus*

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Oxacillin-susceptible, *mecA*-positive *Staphylococcus aureus* isolates create a treatment challenge for the clinician. In this article, we describe two cases of bacteremia from isolates that carried the *mecA* gene but were susceptible to oxacillin (oxacillin-susceptible methicillin-resistant *S. aureus* [OS-MRSA]). DNA microarray analysis was used to characterize these isolates as a *mecA*-positive, clonal complex 5, pediatric strain and a *mecA*-positive, clonal complex 8, USA300 strain.

CASE REPORT

Case 1 was a 53-year-old male who was admitted to the hospital from his adult foster home after successful resuscitation from an asystolic cardiopulmonary arrest. His past medical history included schizophrenia, mental retardation, and recurrent aspiration pneumonia, and he had recently been discharged from the hospital after recovering from an episode of pneumonia. On physical examination, the patient was afebrile, intubated, and required full ventilator support. He was completely unresponsive. His white blood cell count was 27×10^9/liter, with a creatinine concentration of 1.5 mg/dl and a lactate concentration of 6.6 mmol/liter. His blood cultures grew *Staphylococcus aureus*, reported as "presumptive positive for MRSA" (methicillin-resistant *S. aureus*) with an *S. aureus*-specific PCR (BD GeneOhm StaphSR PCR assay; Becton-Dickinson, Sparks, MD), and vancomycin was started. He never regained neurologic function, was transitioned to comfort care, and died 1 week later. Subsequent antibiotic susceptibility testing by Vitek 2 (bioMérieux, Inc., Durham, NC) indicated that the isolate was susceptible to oxacillin, with a MIC of $\leq$0.5 μg/ml.

Case 2 was a 32-year-old male who presented 1 week later with severe left hip pain and 9 days of fevers. He had a past medical history of recurrent urinary tract infections that had been treated with repeated courses of ciprofloxacin. On physical examination, the patient had a temperature of 38.6°C, tenderness to palpation of his left buttock, and left hip pain with passive and active range of motion. Hematologic evaluation demonstrated a white blood cell count of 18×10^9/liter, a hematocrit of 33.8 g/dl, and a platelet count of 210×10^9/liter. His urinalysis had 10 to 25 white blood cells per high-powered field and 3+ bacteria. A computed tomography (CT) scan of the abdomen and pelvis demonstrated an abscess involving the paraspinal muscles, left iliacus, left iliopsoas, left pyriformis, left deep gluteal muscles, and prostate and early septic arthritis of the left sacroiliac joint. The blood culture was reported as "presumptive positive for MRSA" by the same *S. aureus*-specific PCR described above, and vancomycin was started. The patient's urine culture also grew *S. aureus*. Automated phenotypic antibiotic susceptibility testing by Vitek 2, showed both blood and urine isolates to be oxacillin-susceptible methicillin-susceptible *S. aureus* (MSSA) with a MIC of $\leq$0.5 μg/ml and a negative cefoxitin screen. The patient developed a rash after 1 week of therapy with vancomycin, and his treatment was subsequently changed to daptomycin. His blood cultures cleared, and he was discharged from the hospital and completed an 8-week course of antibiotics with full recovery.

Blood cultures from both patients grew *S. aureus* that was identified as MRSA by genotypic testing (PCR), while phenotypic testing (automated broth microdilution, including a cefoxitin screen, oxacillin agar diffusion, and oxacillin Etest) demonstrated MSSA (Table 1). However, two phenotypic test results differed between the two strains: isolate 1 from patient 1 was susceptible to cefoxitin by disk diffusion testing (Kirby-Bauer method according to CLSI guidelines [3]), whereas isolate 2 from patient 2 was resistant. Similarly, isolate 2 grew on a MRSA-specific chromogenic medium, whereas isolate 1 did not (MRSA*Select*; Bio-Rad) (Table 1). Of interest, both isolates failed to grow on the chromogenic MRSA medium of a different company (ChromID MRSA; bioMérieux). The discrepancy between the two different chromogenic media for MRSA is most likely due to subtle differences in the compositions of these selective agar media.

We initially expected the PCR result to be false positive for MRSA due to a so-called "*S. aureus* reverter strain" that had lost the intact *mecA* gene, a phenomenon called the "empty cassette" or "*mecA* dropout" (4, 6). The staphylococcal cassette chromosome SCC*mec* harbors the gene *mecA*, which encodes the penicillin binding protein 2a (PBP2a), which is responsible for methicillin resistance. The BD GeneOhm StaphSR PCR assay targets the *orfX*-SCC*mec* junction that is still present even in a *mecA* dropout strain (2, 4). *mecA* dropout strains can be confirmed with either a negative assay for PBP2a or a negative PCR that directly targets the *mecA* gene. We performed an

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TABLE 1 Phenotypic and genotypic oxacillin and cefoxitin susceptibility testing of *S. aureus* isolates from patients 1 and 2

Test	Result for isolate from[a]:	
	Patient 1	Patient 2
Disk diffusion[b]		
Oxacillin (1 μg)	Sensitive (16 mm)	Sensitive (14 mm)
Cefoxitin (30 μg)	Sensitive (22 mm)	Resistant (19 mm)
Oxacillin MIC by:		
Etest[c]	Sensitive (1 μg/ml)	Sensitive (1 μg/ml)
Vitek 2	Sensitive (≤0.5 μg/ml)	Sensitive (≤0.5 μg/ml)
MRSA PCR[d]	Positive	Positive
PBP2A[e]	Positive	Positive
MRSA*Select* Chromagar (Bio-Rad)[f]	Negative	Positive
ChromID MRSA (bioMérieux)[g]	Negative	Negative

[a] Sensitivity and resistance results are shown in terms of inhibition zone diameter (mm) or MIC (μg/ml).
[b] The disk diffusion assay was performed with Mueller-Hinton agar with a direct colony suspension equivalent to 0.5 McFarland standard (1.5×10^8 colonies), incubated at 35 ± 2°C for 18 h. Oxacillin, sensitive, ≥13 mm, and resistant, ≤10 mm; cefoxitin, sensitive, ≥22 mm, and resistant, ≤21 mm (3).
[c] The Etest MIC for oxacillin was determined with Mueller-Hinton agar with a direct colony suspension equivalent to 0.5 McFarland standard (1.5×10^8 colonies), incubated at 35 ± 2°C for 18 h according to the CLSI recommendations. Sensitive, ≤2 μg/ml; resistant, ≥4 μg/ml) (3).
[d] BD GeneOhm StaphSR, a multiplex PCR targeting the staphylococcal cassette chromosome *mec* (SCC_{mec})-*orfX* junction of *Staphylococcus aureus*.
[e] BinaxNOW PBP2A, an immunochromatographic qualitative assay.
[f] A chromogenic and selective medium from Bio-Rad.
[g] A chromogenic and selective medium from bioMérieux.

TABLE 2 MLST and DNA microarray hybridization profiles of OS-MRSA isolates 1 and 2

Characteristic(s)	Result for:	
	Isolate 1 (patient 1)	Isolate 2 (patient 2)
MLST clonal complex affiliation	Clonal complex 5	Clonal complex 8
Common name	Pediatric clone	USA300
mecA[a]	Positive	Positive
SCC*mec* allotype	SCC*mec* IV	SCC*mec* IV
agrI[b]	Negative	Positive
agrII[b]	Positive	Negative
ACME cluster[c]	Negative	Positive
PVL[d]	Negative	Positive
sek seq[e]	Negative	Positive
seg sei selm seln selo selu egc[f]	Positive	Negative
sea[g]	Positive	Negative
sak chp scn[h]	Positive	Positive
msr(A) *mph*(C) *aphA3*[i]	Negative	Positive

[a] *mecA* is the gene coding for alternate penicillin binding protein 2, defining MRSA.
[b] *agrI* and *agrII*, accessory gene regulator alleles I and II.
[c] ACME, arginine catabolic mobile element.
[d] PVL, Panton-Valentine leukocidin.
[e] *sek* and *seq*, genes coding for enterotoxins K and Q.
[f] *seg* and *sei*, genes coding for enterotoxins G and I; *selm*, *seln*, *selo*, and *selu*, enterotoxin-like genes M, N, O, and U; *egc*, enterotoxin gene cluster.
[g] *sea*, gene coding for enterotoxin A.
[h] *sak*, *chp*, and *scn*, HLB-converting phage genes.
[i] Macrolide resistance genes *msr*(A) and *mph*(C) and *neo* mutant Kan^r gene *aphA3*.

immunochromatographic qualitative assay, Alere PBP2a (Alere, Inc., Waltham, MA), which was positive for both isolates, thereby demonstrating phenotypically that we were not dealing with *mecA* dropout strains.

Both isolates were further genotyped at the Alere Research Laboratory in Jena, Germany. The Alere StaphyType DNA microarray covers 334 target sequences, including species markers, accessory gene regulator (*agr*) alleles, genes encoding virulence factors, capsule type-specific genes, microbial surface component genes, and resistance genes. The protocol and procedures were used as previously described (9–11). Isolates were then assigned to a clonal complex by an automated comparison of hybridization profiles to a collection of reference strains previously characterized by multilocus sequence typing (MLST) (11). The genotype assay confirmed that both strains were indeed $mecA^+$ MRSA strains, with isolate 1 assigned to the clonal complex 5 (CC5) pediatric clone and isolate 2 demonstrating characteristics of the clonal complex 8 (CC8), Panton-Valentine leukocidin (PVL)-positive USA300 strain.

Isolate 1, the CC5 MRSA pediatric clone, contained a number of enterotoxin genes and a β-hemolysin (HLB)-converting phage. This strain has been described in cystic fibrosis patients (14) as well as pediatric populations throughout the United States, Portugal, Argentina, and Colombia (13). Isolate 2 was the USA300 strain, which is widespread throughout the United States and is often community associated (12). This isolate contained the ACME (arginine catabolic mobile element) locus, which is hypothesized to enhance the organism's survival on intact skin (9), an HLB-converting phage, and enterotoxin genes, as well as macrolide and aminoglycoside resistance genes *msr*(A), *mph*(C), and *aphA3*, respectively. The genotypes, resistance genes, and toxin-related genes are summarized in Table 2.

These cases describe two unique bloodstream isolates of *S. aureus* that were *mecA* positive yet oxacillin susceptible (OS-MRSA). The current definition of CLSI for methicillin-resistant *Staphylococcus aureus* is that the isolate has an oxacillin MIC of ≥4 mg/liter and/or contains the *mecA* gene or its gene product, PBP2a (3). However, as described in this report, *S. aureus* strains expressing *mecA* may exhibit oxacillin MICs that are in the susceptible range. These isolates have previously been described with heteroresistance proposed as an explanation for the discrepancy between the phenotype and genotype (1, 15). Hososaka et al. described six OS-MRSA isolates in Japan, with two-thirds of the isolates having SCC*mec* type III and none of the isolates containing PVL or enterotoxin (7). *In vitro* and *in vivo* studies have confirmed that OS-MRSA isolates that contain *mecA* and express PBP2a are functionally oxacillin resistant in time-kill curves and in a mouse model with experimental thigh infections (8, 15). This raises the important question of how often are these strains missed in the clinical laboratory? Most *S. aureus* strains are identified as MSSA or MRSA based on phenotypic susceptibility testing alone. Two studies in which consecutive *S. aureus* strains were analyzed phenotypically and genotypically both showed that about 1% of *mecA*-positive *S. aureus* strains were oxacillin susceptible (7, 15). In view of the tens of thousands of *S. aureus* infections in the United States each year, even a low prevalence may result in a significant number of MRSA isolates being misclassified as MSSA. Unexpressed *mecA* might be induced upon exposure to β-lactams

(i.e., during therapy) and could be a cause for therapeutic failures. Thus, isolates from cases in which a β-lactam therapy failed should be scrutinized for the possible presence of *mecA*, even if initial tests indicated susceptibility, as suggested by Sakoulas et al. in 2001 (15). Alternatively, laboratories could consider routine testing of *mecA* or PBP2a in all *S. aureus* isolates that cause serious, invasive infections, such as staphylococcal bacteremia, to prevent possible treatment failures with β-lactam therapy due to OS-MRSA.

Until recently, tests for *mecA* or its gene product, PBP2a, were considered to be the most accurate methods for prediction of resistance to oxacillin (3). This seems to have come into question by the recent discovery of MRSA strains that are apparently *mecA* and PBP2a negative, although they contain a highly divergent *mecA* gene, a putative ancestral *mecA* homolog called $mecA_{LGA251}$ (5, 16). In conclusion, our cases demonstrate that with increased use of genotypic susceptibility testing in bacteriology, we are going to see more discrepancies between phenotypic and genotypic test results. Each method comes with its own set of limitations and challenges. Both approaches complement each other and together will help us paint a more granular picture of the multiple ways bacteria outsmart us.

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